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EXHIBIT A

A method for the simultaneous amplification and subsequent simultaneous detection of a first

target DNA and a second target DNA comprising:

- A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:
- i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to third and fourth primers which are 400 nucleotides, specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a T_m within the range of from 65 to 74°C, as calculated by the formula $T_m(^{\circ}C) = 67.5 + 0.34 (% G + C) - 395/N$, all of said primer $T_{m}{}^{\prime}{}s$ being within about $5^{\circ}C$ of each other, said first and second primers having nucleotide lengths which differ from each other by no more nucleotides, and said third and fourth primers having

nucleotide lengths which differ from each other by no more than 5 nucleotides, and

- ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, each of priming and primer extension are carried out at a temperature within the range of from 62 to 75°C,
- B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.
- 36. [Amended] A method for the simultaneous amplification and <u>subsequent simultaneous</u> detection of a first target DNA and a second target DNA comprising:
- A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:
 - i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a T_m within the range of from 67 to 74°C, all of said primer $T_{m}{}^{\prime}s$ being within about 2°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

- ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from 62 to 75°C,
- B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.